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Roles of TIF1β Phosphorylation in Colorectal Cancer

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内容記述	この博士論文は内容の要約のみの公開（または一部非公開）になっています
year	2017
その他のタイトル	大腸がんにおけるTIF1βのリン酸化の役割
学位授与大学	筑波大学 (University of Tsukuba)
学位授与年度	2017
報告番号	12102甲第8398号
URL	http://hdl.handle.net/2241/00152249

School of Integrative and Global Majors
Ph.D. Program in Human Biology (HBP)

論文概要

Dissertation Abstract

The title of Doctor Dissertation:

Roles of TIF1β Phosphorylation in Colorectal Cancer

大腸がんにおける TIF1βのリン酸化の役割

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Abstract

1. Project I

PP44 dephosphorylates TIF1β in colorectal cancer

1.1. Background Protein phosphatase 4 is a serine/threonine phosphatase that has critical functions in DNA double-strand break repair and cell cycle by the regulation of phosphorylation of its target proteins. TIF1β is an intermediary factor of transcription and epigenetic modulator of gene expression in several physiological processes. However, gaps exist in how PP4C functions in TIF1β phosphorylation in cancer.

1.2. Purpose To elucidate the biological correlation of TIF1β phosphorylation with PP4 in the tumor

1.3. Materials and Methods

i、 The expression of the PP4C protein and the phosphorylation level of TIF1β were detected by immunohistochemistry staining and immunohistochemistry multiple-staining on CRC with PP4C, total TIF1β, and phospho-TIF1β antibodies.

ii、 The upstream signaling pathway of TIF1β phosphorylation was examined under EGF induction with kinases inhibitors (AG1478 and U0126) and analyzed by Western blot.

iii、 PP4C regulated dephosphorylation of TIF1β at Ser473 was examined under EGF induction with transient PP4C expression in HEK293T by Western blot and in vitro phosphatase assay. Also, it was further confirmed by PP4C expressing cells under EGF induction compared with control cells.

iv、 The functions of PP4C were evaluated by cell proliferation assay, colony formation assay, sphere formation assay and *in vivo* tumor formation assay.

v、 To examine the potential of anticancer drug resistance in PP4C expressing cells, cells were treated with anticancer drugs. Cell survival was measured by MTT assay.

1.4. Result

i、 High expression of PP4C has found in high grade of CRC tissues

ii、 The phosphorylation level of TIF1β has a negative correlation with PP4C in CRC

iii、 EGF induces TIF1β phosphorylation through ERK signaling pathway

iv、 PP4C dephosphorylates TIF1β phosphorylation

v、 PP4C suppresses proliferation and tumorigenesis and increases anticancer drug resistance

1.5. Discussion In this study, we show that there is a negative correlation between PP4C expression and TIF1β phosphorylation in CRC. TIF1β phosphorylation is transiently induced through ERK signaling pathway and is suppressed in the presence of PP4C. In addition, PP4 suppresses cell proliferation, tumor growth and promotes anticancer drug resistance. Our finding provides evidence supporting that TIF1β can be post-translationally modified upon growth signal in cancer, and the potential value of PP4C in the regulation of TIF1β phosphorylation on CRC aggressiveness suggesting that inhibition of PP4C may be an option to enhance the chemotherapeutic effect of conventional anticancer drugs toward CRC.

2. Project II Oxidative Stress-induced phosphorylation in TIF1β

2.1. Background Reactive oxygen species (ROS) cause significant damage to macromolecules including DNA. One of ROS, hydrogen peroxide, is thought to be a signal molecular in the growth of tumor cells. TIF1β Phosphorylation is required for an efficient DNA repair and cell survival. However, the effect of oxidative stress on TIF1β on the biological behaviors of colorectal cancer (CRC) cells have not been determined.

2.2. Purpose of investigating the effect of oxidative stress on TIF1β in CRC cells

2.3. Materials and Methods The effect of H_2O_2 and the kinases responsible on TIF1β was examined by H_2O_2 stimulation and analyzed by western blot.

2.4. Result Phosphorylation of TIF1β is induced by hydrogen peroxide and mediated through MAPKs.

2.5. Discussion In this study, we reported that H_2O_2 induces phosphorylation of TIF1β. Also, H_2O_2 activated p42/44 MAPKs and p38 MAPKs suggested that the oxidative stress induced TIF1β phosphorylation through the mediators, p42/44 MAPKs and p38 MAPKs, in colorectal cancer cells.

2.6. Summary This is the first study directly demonstrating that TIF1β can be post-translationally modified upon growth signal in cancer cells. Interestingly, PP4C may also regulate TIF1β-Ser473 phosphorylation under oxidative stress in response to DNA damage. In this dissertation, we demonstrated that pTIF1β-Ser473 are transiently induced upon growth factor induction and under oxidative stress. According to our results, TIF1β phosphorylation is likely mediated by the cooperation of p42/44 or p38 MAPKs and other kinases not identified in response to growth factor and oxidative stress in different perspectives on colorectal cancer.